

IN THE CLAIMS:

Please cancel Claims 1-18, 43 and 52, without prejudice to or disclaimer of the subject matter therein. Please add the following new Claims 61-80.

61. A method to produce glucosamine by fermentation, comprising:

a. culturing in a fermentation medium comprising an inducing compound that regulates a *lac* promoter, a microorganism which is transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a glucosamine-6-phosphate synthase which has glucosamine-6-phosphate synthase activity, and a recombinant nucleic acid molecule comprising a *lac* promoter; wherein expression of said nucleic acid sequence a glucosamine-6-phosphate synthase is regulated through induction of said *lac* promoter;

wherein said step of culturing produces and accumulates a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and

b. recovering and purifying said product.

62. The method of Claim 61, wherein said nucleic acid sequence encoding a glucosamine-6-phosphate synthase is operatively linked to a T7 promoter or to a T7-*lac* promoter; and wherein said microorganism is further transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a T7 RNA polymerase, said sequence being operatively linked to said *lac* promoter;

wherein induction of said *lac* promoter with said compound regulates said T7 promoter or T7-*lac* promoter and expression of said glucosamine-6-phosphate synthase.

63. The method of Claim 61, wherein said *lac* promoter is the *lacUV5* promoter.

64. The method of Claim 61, wherein said inducing compound is isopropylthio- β -D-galactopyranoside (IPTG).

65. The method of Claim 61, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence encoding amino acid sequence SEQ ID NO:16.

66. The method of Claim 61, wherein said nucleic acid sequence encoding a glucosamine-6-phosphate synthase comprises a genetic modification that results in at least one nucleic acid modification selected from the group consisting of deletion, insertion, and substitution of at least one nucleotide of said nucleic acid sequence encoding glucosamine-6-phosphate synthase, said at least one nucleotide modification resulting in increased glucosamine-6-phosphate synthase activity.

67. The method of Claim 61, wherein said recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase comprises a genetic modification which reduces glucosamine-6-phosphate product inhibition of said glucosamine-6-phosphate synthase.

68. The method of Claim 61, wherein said at least one nucleic acid modification results in an amino acid modification at an amino acid sequence position, corresponding to amino acid sequence SEQ ID NO:16, selected from the group consisting of Ile(4), Ile(272), Ser(450), Ala(39), Arg(250), Gly(472), Leu(469), and combinations thereof.

69. The method of Claim 61, wherein said recombinant nucleic acid molecule is integrated into the genome of said microorganism.

70. The method of Claim 61, wherein said microorganism has at least one additional genetic modification in a gene encoding a protein selected from the group consisting of *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, *N*-acetyl-glucosamine-specific enzyme II^{Nag}, phosphoglucosamine mutase, glucosamine-1-phosphate acetyltransferase-*N*-acetylglucosamine-1-phosphate uridyltransferase, phosphofructokinase, Enzyme II^{Glc} of the PEP:glucose PTS, and EIIM.P/III^{Man} of the PEP:mannose PTS, wherein said genetic modification decreases the activity of said protein.

71. The method of Claim 61, wherein said microorganism has additional modifications in genes encoding the following proteins: *N*-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase and *N*-acetyl-glucosamine-specific enzyme II^{Nag}.

wherein said genetic modification decreases the activity of said proteins.

72. The method of Claim 61, wherein said microorganism is selected from the group consisting of bacteria and yeast.

73. A method to produce glucosamine by fermentation, comprising:

a. culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, a microorganism which comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase, wherein said genetic modification is selected from the group consisting of:

i. transformation of said microorganism with a recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase; and

ii. genetic modification of a gene encoding glucosamine-6-phosphate synthase that increases the activity of said glucosamine-6-phosphate synthase, wherein said genetic modification results in at least one nucleic acid modification selected from the group consisting of deletion, insertion, and substitution of at least one nucleotide of said gene encoding glucosamine-6-phosphate synthase, said at least one nucleotide modification resulting in increased glucosamine-6-phosphate synthase activity;

wherein said microorganism further comprises at least one additional genetic modification in a gene encoding a phosphatase, wherein said genetic modification increases the activity of said phosphatase;

wherein said step of culturing produces and accumulates a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and

b. recovering and purifying said product.

74. The method of Claim 73, wherein said phosphatase is alkaline phosphatase.

75. The method of Claim 73, wherein expression of said recombinant nucleic acid molecule is regulated through induction of a *lac* promoter, and wherein said fermentation medium further comprises an inducing compound that regulates the *lac* promoter.

76. The method of Claim 73, wherein said recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase comprises a genetic modification which increases the activity of said glucosamine-6-phosphate synthase.

77. The method of Claim 73, wherein said step of culturing is at a temperature of from about 20°C to about 40°C.

78. A method to produce glucosamine by fermentation, comprising:

a. culturing in a fermentation medium comprising assimilable sources of carbon, nitrogen and phosphate, and at a temperature of from about 20°C to about 40°C, a microorganism which comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase, wherein said genetic modification is selected from the group consisting of:

i. transformation of said microorganism with a recombinant nucleic acid molecule encoding glucosamine-6-phosphate synthase; and

ii. genetic modification of a gene encoding glucosamine-6-phosphate synthase that increases the activity of said glucosamine-6-phosphate synthase, wherein said genetic modification results in at least one nucleic acid modification selected from the group consisting of deletion, insertion, and substitution of at least one nucleotide of said gene encoding glucosamine-6-phosphate synthase, said at least one nucleotide modification resulting in increased glucosamine-6-phosphate synthase activity;

wherein said step of culturing produces and accumulates a product selected from the group consisting of glucosamine-6-phosphate and glucosamine from said microorganism; and

b. recovering and purifying said product.

79. The method of Claim 78, wherein said temperature is from about 25°C to about 40°C.

80. The method of Claim 78, wherein said microorganism further comprises at least one additional genetic modification in a gene encoding alkaline phosphatase, wherein said genetic modification increases the activity of said alkaline phosphatase.